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IMPROVED OVULATION RATE AND IMPLANTATION IN RATS TREATED WITH ROYAL JELLY.

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ABSTRACT

The ovaries and uteris of 12 mature female rats (Rattus norvegicus) were examined to determine the effect of commercial royal jelly on ovulation, ovarian weight and implantation rates. Rats were split in two groups of 6 each. Group one served as the treatment and group two the control. A daily dose of 25mg of royal jelly was administered orally for 26 days under standard laboratory conditions. Approximately ten days post copulation, rats were euthanized with chloroform and ovulation was determined by the number of corpora lutea present in the ovaries. Ovarian weight was recorded and implantation was determined based on the number of implants present in the uterus. The mean number of corpora lutea found in experimental and control groups were 8.0±1.8 and 5.3±1.5 respectively. Mean ovarian weight in treated and control groups were 0.160g±0.035 and 0.078g±0.02 respectively. Percentage implantation rates of treated and control groups were 93.33% and 90.62% respectively The study revealed that royal jelly can significantly improve the number of ova released per estrus cycle ($p<0.05$) and the ovarian weight ($p<0.05$) of mature female rats. Royal jelly may be effective in improving ovulation and implantation in rats.

Key words: Royal jelly, Ovulation, Implantation, *Rattus norvegicus*

INTRODUCTION

Royal jelly (RJ) from honey bees, *Apis melifera* is an extremely nutritious substance secreted by the hypopharyngeal glands of the nurse bees (Isidorova *et al.*, 2009). It contains an array of nutrients including crude proteins, b-complex vitamins, water, essential amino acids, sugars and fatty acids as well as antibiotic and anti-oxidizing components amongst others (Sugiyama *et al*., 2012).

In bee colonies RJ is primarily for maintaining the queen bee and feeding larvae. This nutrient rich substance is believed to be the key substance that accounts for the fecundity and long life of the queen bee (Webb, 2011). Suzuki et al (2008) reported that RJ has weak estrogenic activity in rats. In their study rats treated subcutaneously with compounds isolated from RJ elicited mild hypertrophy of the luminal epithelium with no increase in uterine weight. Several other studies have suggested that RJ may improve pregnancy rates in rabbits, ewes and sheep (Kridli and Al-Khetib, 2006; Husein and Kridli, 2002; Khattab et al., 1989; Csuka et al., 1978).
Studies investigating the effect of RJ on ovulation, ovarian weight and implantation

rates are limited. We seek to examine the fertility improving potential of commercial RJ (Forever Living Co. Inc). The present study exposes mature *Rattus norvegicus* to 25mg of commercial royal jelly daily for 26 days and reports changes in ovulation, ovarian weights and implantation rates.

MATERIAL AND METHODS

Animals and experimental design

Twelve female *Rattus norvegicus* (55days old) weighing 180 to 200g were randomly divided equally into two groups. The experiment was conducted at the Majors laboratory of the Department of Animal and Environmental Biology, University of Port Harcourt. Two males were kept in a separate area away from the females in order to ensure mating is effected when paired. The animals were paired in confinement made up of a wide plastic basin, wire gauze roofing, and grand wood shavings as beddings. Both groups were fed normally with rat feed and water. Each rat in the experimental group was administered 25mg/day of commercial RJ orally for 26 days until they were killed. The control group was administered 5ml of water concurrent with other treatment for the same period.

Royal jelly

Commercial royal jelly produced by Forever Living Co. Inc. was purchased locally and used for this experiment. The specific composition of royal jelly as stated by the manufacturer are: purified natural royal jelly; sorbitol; fructose; natural orange flavor; stearic acid; magnesium stearate and silica.

Determination of estrous stage and mating of animals

Estrus was verified according to the method of Marcondes *et al*., 2002. Vaginal secretions to determine estrus stage were collected one week after commencement of the treatment. Once estrus stage was confirmed a female was paired with the male for 24 hours in a separate unit for mating to occur. Mating was verified from examination of vaginal smears.

Examination of ovaries and uterus

Ten days after mating the females were euthanized using chloroform in a desiccator and dissected for the examination of the ovaries and uterus. The ovaries were extracted, and weighed using Mettler balance (Mettler Toledo^{TM}). Ovulation was determined by the number of corpus luteum found in the ovaries. The number of implants found during examination of the uterus was recorded.

Statistical analysis

The results of the experiment were expressed as mean ±S.D. Statistical analysis was done using a two-tailed t-test in Microsoft Excel 2013 (Microsoft 2013) and differences was considered statistically significant if $p \leq 0.05$.

RESULTS

Ovulation and implantation rates in rats treated with commercial royal jelly

The effect of royal jelly on ovulation and implantation in mature female rats is shown in Table 1. Ovulation in mature female rats treated orally with a daily dose of 25mg of RJ for 26 days was significantly different ($p < 0.02$) from those of the control group.

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Table 1: Number of ova released in experimental and control groups

Animal No.	Treatment		Control	
	No. of Corpora lutea	No. of Implants	No. of Corpora lutea	No. of Implants
1	10	10	8	8
2	7	7	6	6
3	9	8	6	6
4	5	5	4	1
5	7	7	4	5
6	10	8	4	4
∑x	48	45	32	30
Mean±S.D	8.00±1.8	7.5±1.5	5.33±1.5	5±2.2

The number of ova released was compared with the number of implantations present in the uterus of experimental and control groups. Rats treated with RJ recorded a mean implantation rate of 93.3% and the control group had a mean implantation rate of 90.62%. The difference between the two groups were not statistically significant.

Effect of royal jelly on ovarian weight

The effect of RJ on ovarian weight is shown in Table 2. The difference between the means of the two groups was statistically significant at ($p < 0.05$).

Table 2: Ovarian weight of experimental and control groups

Replicate	Treatment (g)	Control (g)
1	0.220	0.109
2	0.165	0.094
3	0.155	0.071
4	0.161	0.083
5	0.160	0.061
6	0.100	0.050
∑x	0.961	0.468
Mean±S.D	0.160±0.035	0.078±0.02

DISCUSSION

Royal jelly does have estrogenic activity as previously reported (Mishima et al., 2005; Suzuki et al., 2008). Our results support previous reports that royal jelly is capable of producing greater pregnancy in treatments than in controls. In addition to increasing lambing rates in ewes (Kridli and Al-Ketib, 2005), our study further suggests that at low doses, royal jelly can improve fertility in mature *Rattus norvegicus* by significantly increasing ($p < 0.05$) the number of ova released per reproductive cycle. Specific components unique to royal jelly - trans-10-hydroxy-2-decenoic acid and 10-hydroxydecanoic acid (Terada *et al.,* 2011) - like other exogenous estrogens, can elicit estrogen like effects through interactions with estrogen receptors (Yang *et al.,* 2012). This could account for the improved fertility recorded in animal studies of royal jelly.

Rats treated with commercial royal jelly had ovaries that weighed significantly higher ($p<0.01$) than controls. A significantly higher ovarian volume has been positively associated with pre-menopausal women (Pavlik et al., 2000). In fact, Pavlik *et al*., 2000 reported that younger women (<30 years) had a significantly higher ovarian volume than older women. Other studies on cows, calves and heifers have reported that higher ovarian weights have been positively correlated with increased ovarian activity in a given ovary (Hammond, 2014). The ovarian weight can largely be attributed to the relatively larger amounts of corpora lutea and /or follicles present in the ovaries (Hammond, 2014). It is apparent that royal jelly can improve the capacities and performance of mammalian ovaries.

Implantation is a complex physiological process that is largely influenced by the hormonal input of the corpus luteum (Tomac *et al.,* 2011). While progesterone secreted by the corpus luteum is essential for establishment of pregnancy, it is very crucial in embryo implantation. In the present study, mean implantation loss was less in RJ treated groups (6.67%) than in controls (9.38%). Nevertheless, the likelihood of some grafian follicles luteinizing without discharging ova, and being mistaken for corpora lutea cannot be overlooked. This may compromise the accuracy of implantation estimates. Further studies involving a higher dose of RJ and a larger sample size would allow for a more comprehensive investigation of the role of RJ in embryo implantation.

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